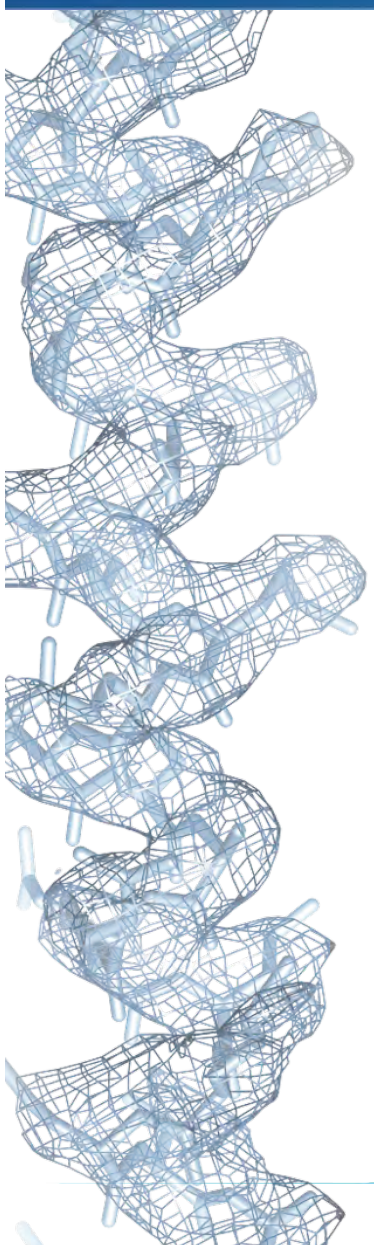


Recent Advances in Cryo-Electron Microscopy:

Opportunities and New Frontiers

Sriram Subramaniam
National Cancer Institute

February 3, 2015

Outline of presentation

Highlights of December 2014 workshop and recommendations

- Introduction to cryo-EM
- Specific topics addressed at workshop
- Major challenges to growth of cryo-EM in the US and possible solutions

A possible role for the FNLCR in advancing cryo-EM

- The gap in "structural biochemistry"
- Examples of cancer targets and challenges that lie ahead
- Unique opportunities at FNLCR for cryo-EM in cancer structural biology
- A phased execution plan and suggested budget

Methods for protein structure determination

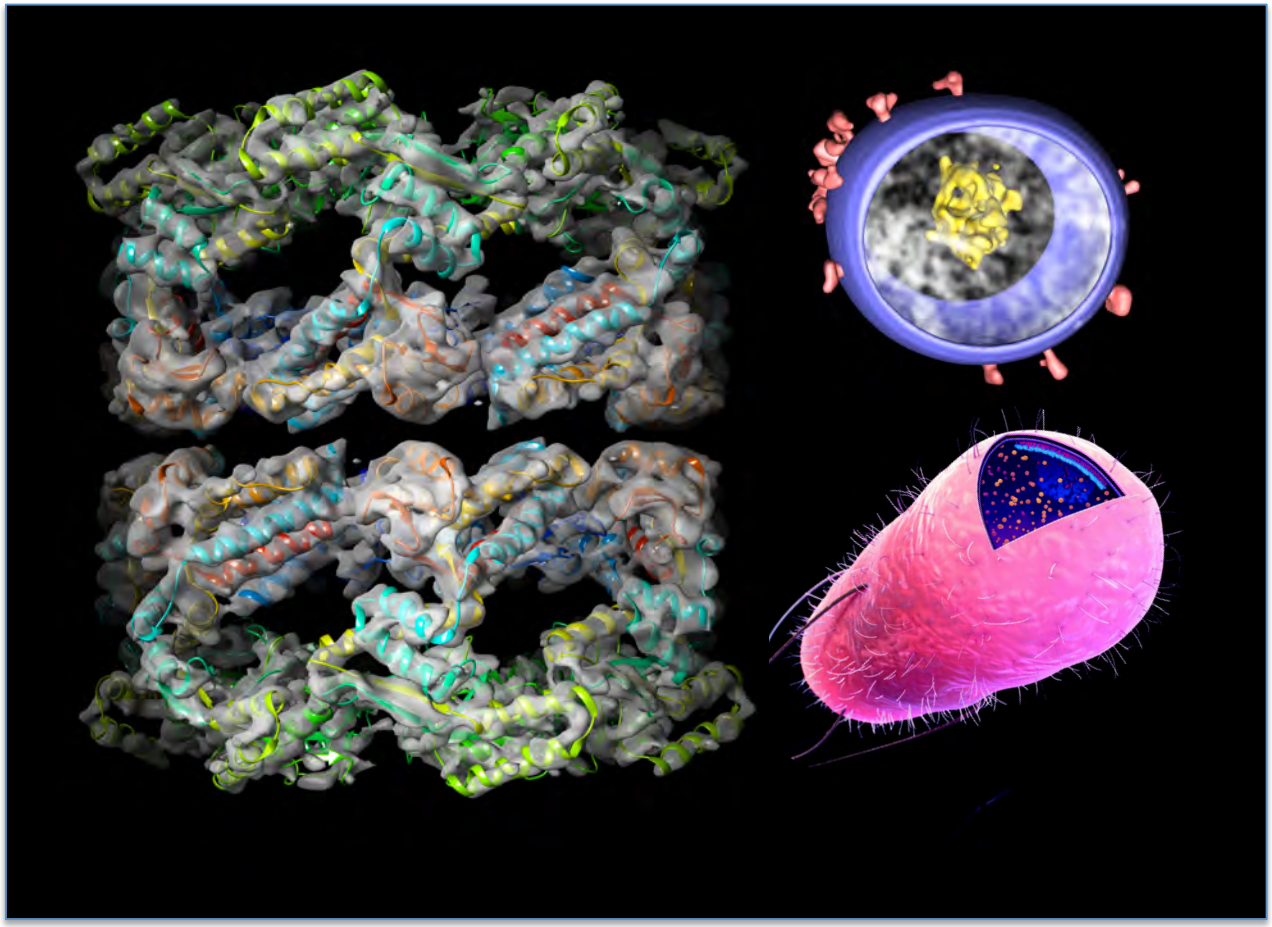
X-ray crystallography

- Is presently dominant method for structure determination of biological macromolecules
- Needs well-ordered 3D crystals; flexible regions of proteins typically need to be either truncated or modified; structure in crystal may not reflect solution structure

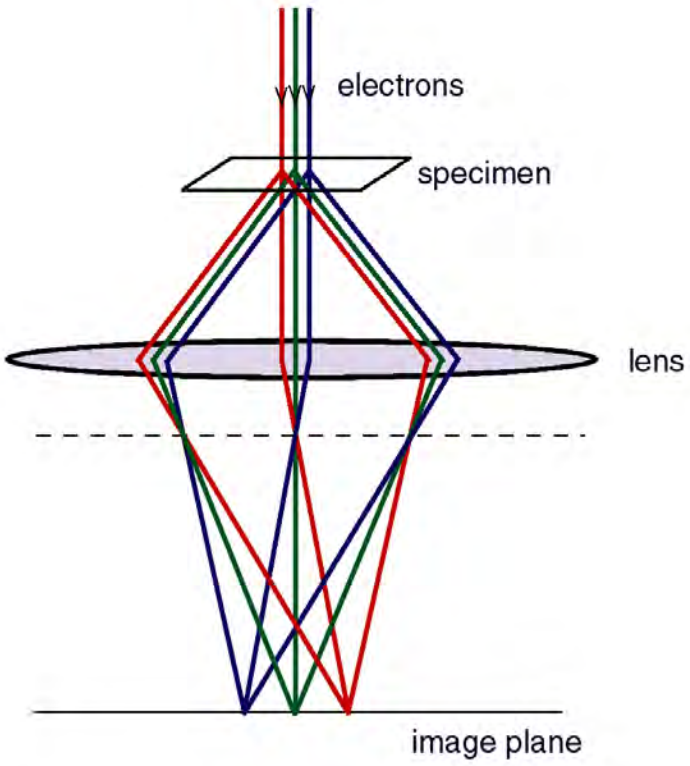
NMR spectroscopy

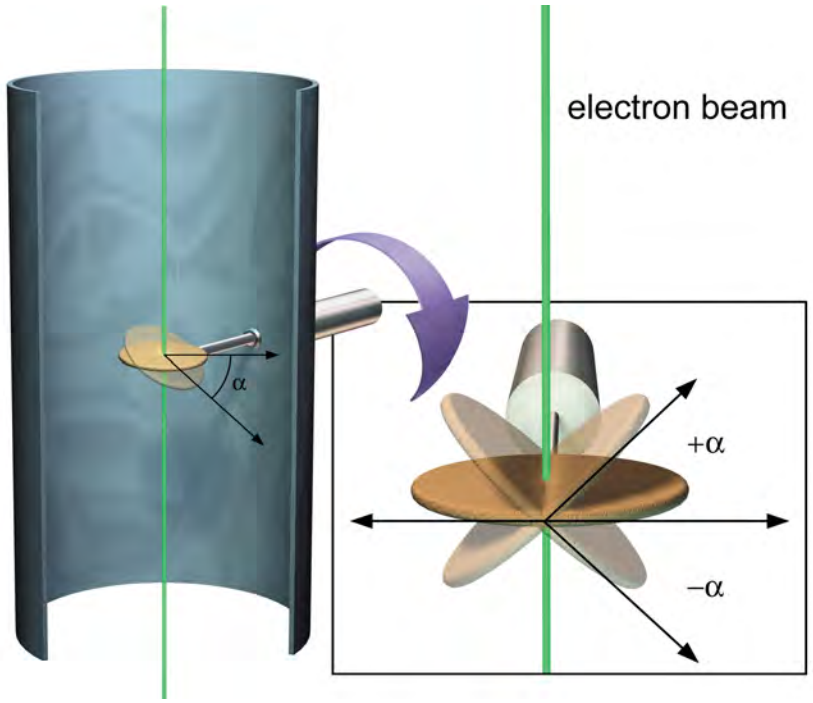
- Enables structure determination in solution, providing important information on dynamics
- Limited primarily to small proteins (typically < 50 kD)

Cryo-EM: new tools for determining 3D structures of cells, viruses and molecules



Principle of 2D and 3D imaging with electrons





The emergence of atomic resolution cryo-EM

1990: First atomic resolution model from electron crystallography of 2D protein crystals (3.5 Å)

1995: Articulation of prospects of obtaining atomic resolution protein structures *without* crystals

2008: First near-atomic resolution icosahedral viral structures (3.9 Å)

2013: First near-atomic resolution membrane protein structure (3.4 Å)

2013-2014: Numerous structures reported at resolutions between 3.0 Å and 4.0 Å

Henderson, Quart. Rev. Biophys. (1995)

The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules

RICHARD HENDERSON
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Topics covered at the December workshop

Session I – Recent progress in high-resolution biological electron microscopy

Session II – Cryo-EM and X-ray crystallography: Value of hybrid approaches

Session III – Need for centers with advanced cryo-EM instrumentation

Session IV – NIH perspective and participation of broader research community

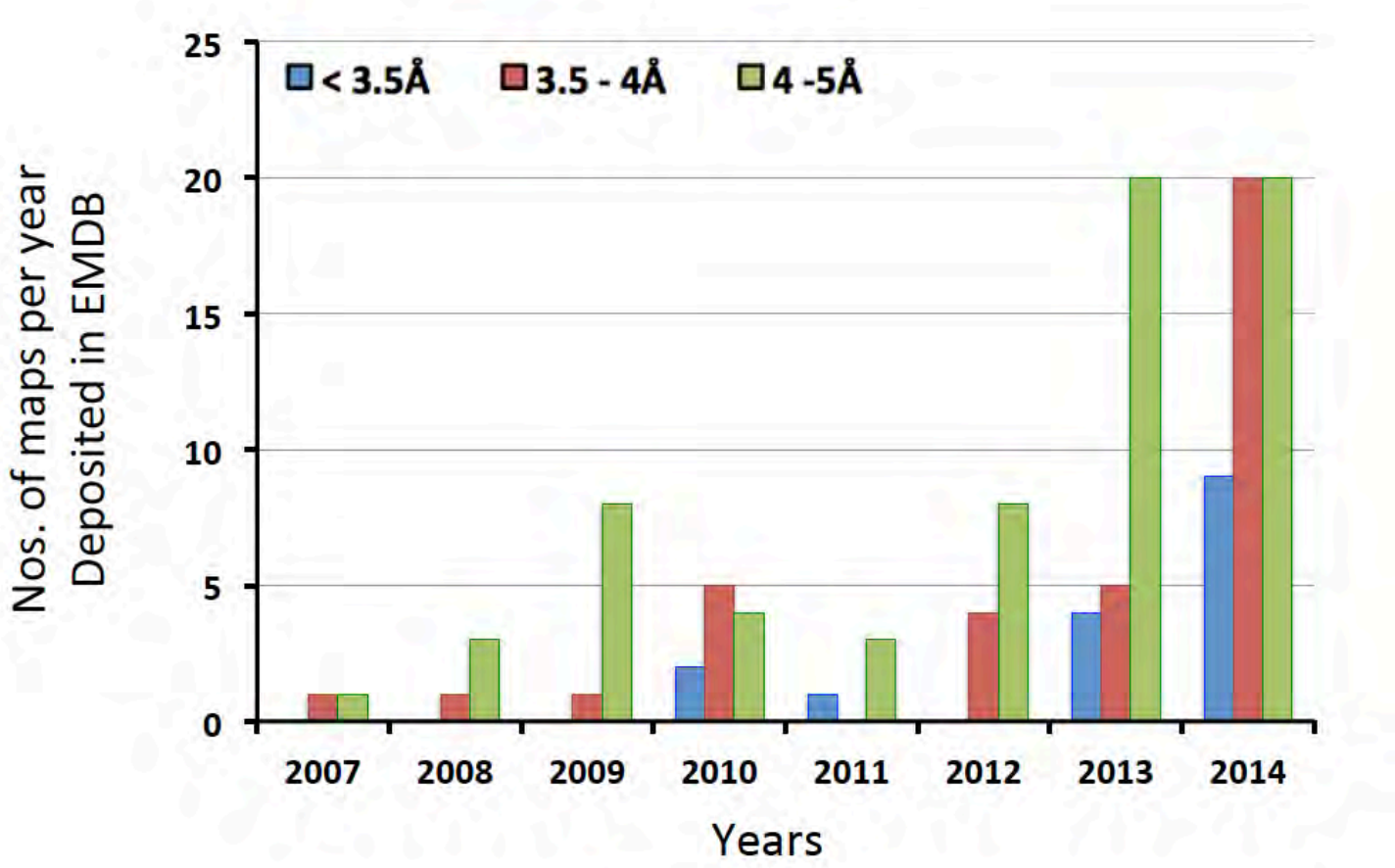
Some of the questions addressed:

- **What has changed in the cryo-EM field over the last year? What is the significance of the new advances?**
- **What is the impact for structural biologists who are not cryo-EM specialists?**
- **What is the impact for advancing study of biological mechanisms?**
- **What are the bottlenecks to the broader dissemination of this technology?**
- **Are the methods developed to a point where it is ready for use as a routine tool?**
- **How do the new advances in cryo-EM impact those who presently use X-ray crystallography and NMR as their principal structural tools?**
- **What are the practical challenges that structural and cell biologists face in having routine access to cryo-EM technology?**
- **What are the pros and cons of having local access vs. remote access to advanced cryo-EM instrumentation?**
- **What are the bottlenecks to getting trained users in X-ray and NMR labs?**

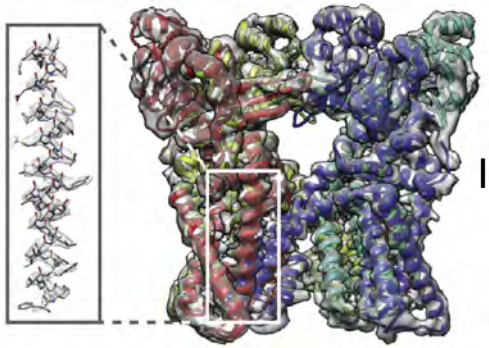
Some of the questions addressed:

- **Is the "synchrotron" model applicable to cryo-EM?**
- **What are the ancillary needs for a cryo-EM center in order for it be effective? What types of activities should be central to the mission?**
- **What are the strengths and pitfalls of a single DOE-style national lab that specializes in cryo-EM vs. having many centers distributed across the country?**
- **What can an institution such as the FNLCR contribute to supporting a national cryo-EM effort in ways that other places cannot?**
- **How might different Institutes join forces to prevent duplication and make NIH support for cryo-EM more effective?**
- **How can various interested extramural communities be supported and engaged in a fair and transparent manner?**
- **What are good approaches for companies with expertise in computing to engage and support cryo-EM computing needs?**
- **Is supporting cryo-EM at FNLCR an activity that is sustainable on a long-term basis?**

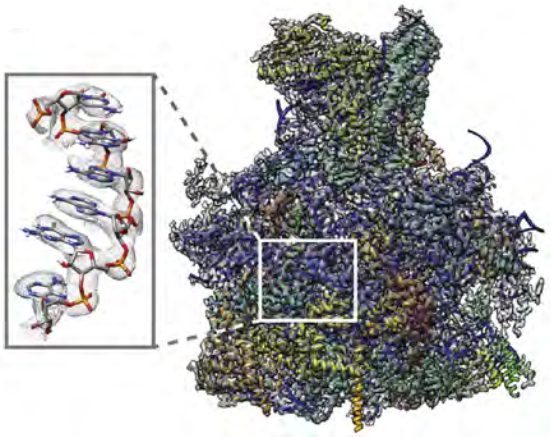
Recent growth of cryo-EM depositions



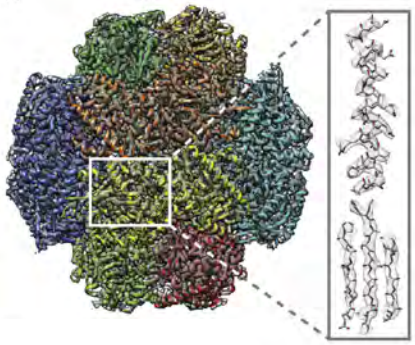
Some examples



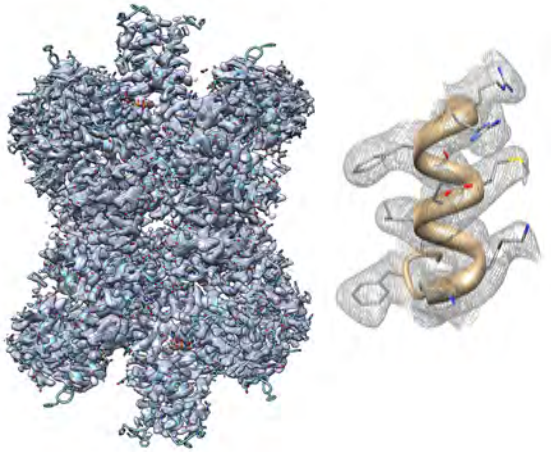
Ion channel
(UCSF)
3.4 Å



Ribosome
(MRC Cambridge)
3.2 Å

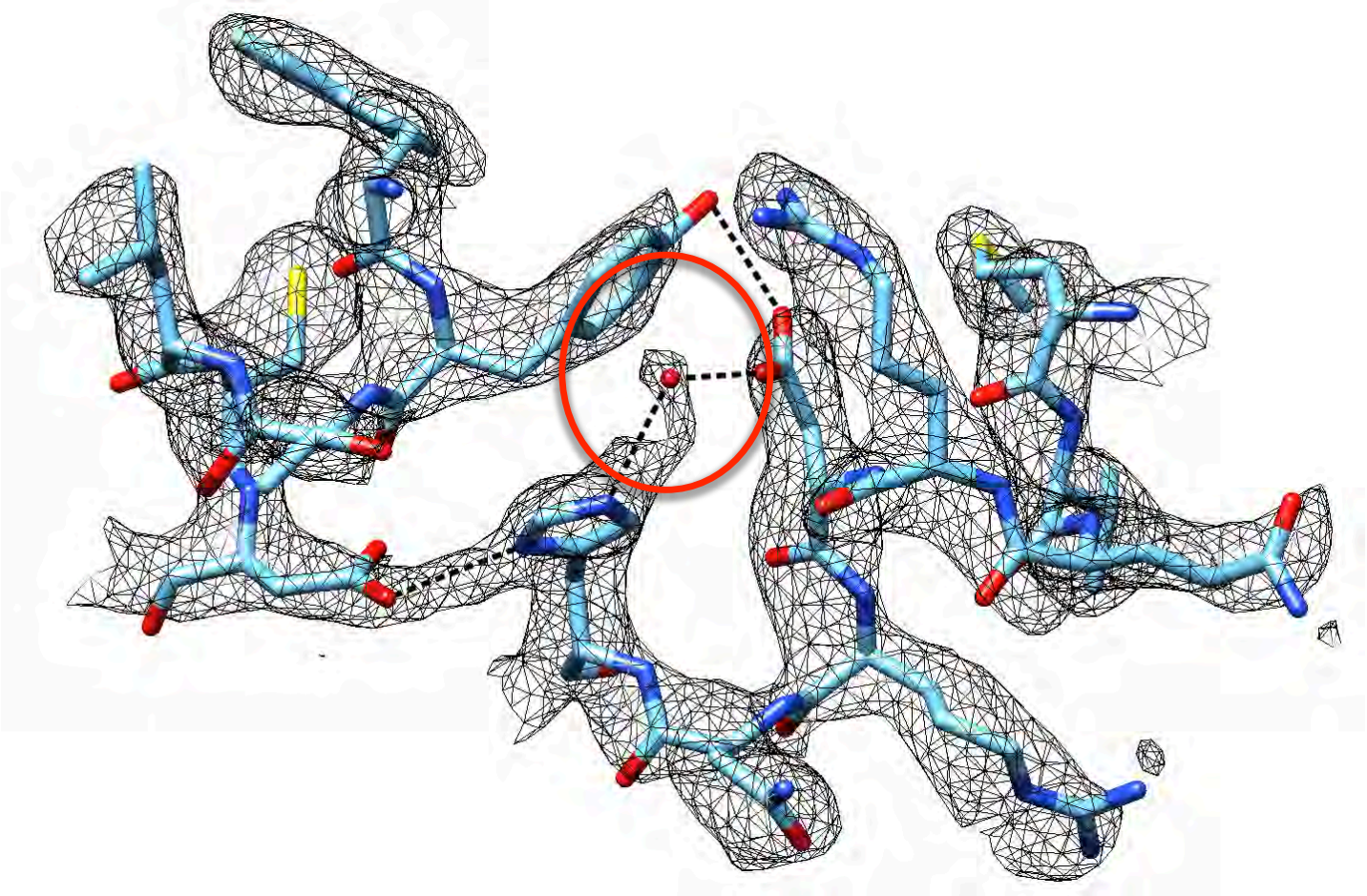


Hydrogenase
(Max-Planck)
3.3 Å



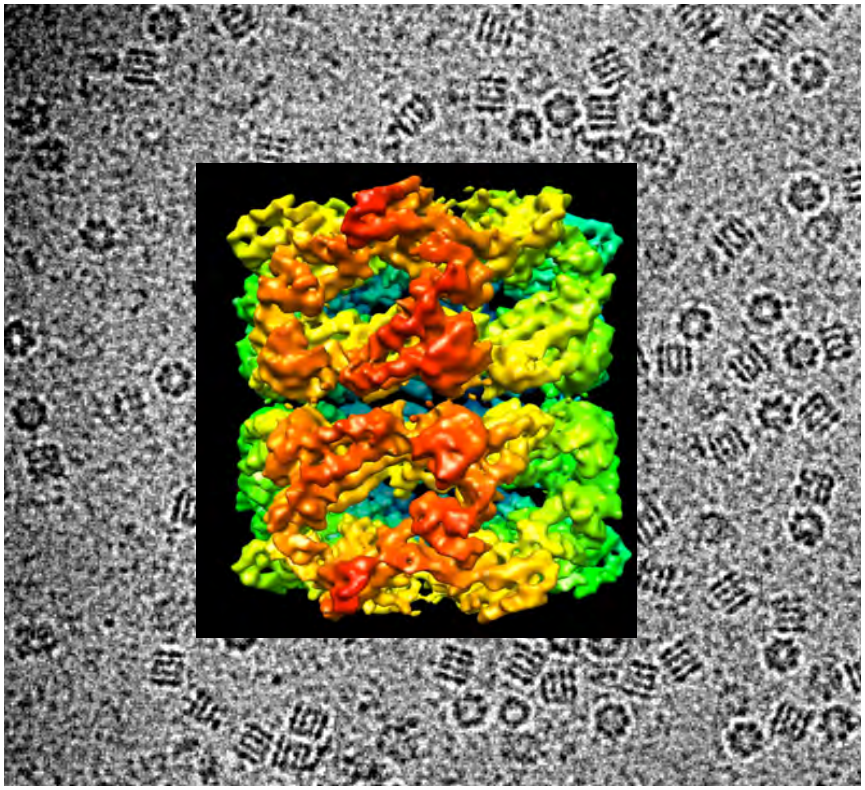
Metabolic enzyme
(NCI)
3.0 Å

Visualization of active sites and water molecules



A simplified view of steps in structure determination

A simplified view of steps in structure determination



Summary of significant technical advances

- Direct electron detectors
- Publicly accessible software
- Better tools for validation
- Structures of dynamic proteins in different states
- Fewer images, higher throughout with new technologies
- Can solve structures intractable by X-ray crystallography
- …but methods are not yet developed for general use

Major challenges to growth of cryo-EM in the US

- Lack of adequate access of labs with expertise in cryo-EM to advanced instrumentation
- Not enough numbers of scientists trained in all aspects of cryo-EM structure determination
- Insufficient access of labs with expertise in X-ray and NMR methods to specific cryo-EM expertise and eventually to advanced instrumentation
- Outdated instrumentation at most institutions and lack of adequate funding for upgrading to modern equipment

Main recommendations from NCI workshop

National cryo-EM facilities:

- Set up ~ 3 user-friendly state-of-the art national cryo-EM facilities with highest-end instruments and detectors. Location at synchrotron sites would be a good strategy. Allocated budgets should also cover salaries of staff and service contracts.

- Recently set up national cryo-EM facility at the UK Diamond synchrotron is a good model

Localized cryo-EM facilities

- Recommend supporting a substantial number of Universities and Institutes spread throughout the US with more modern equipment with matching support from host institution for costs of instrument purchase, service contracts and support staff. The New York Structural Biology Center is a good model for a localized cryo-EM facility.

Retraining of structural biologists:

- Recommend a robust training program to introduce X-ray, NMR and biological investigators to cryo-EM and tomography.

NIGMS report recommendation (December 2014)

- It is recommended that NIGMS support regionally-shared resources with the aim of eventually providing access to any researcher whose project would benefit from structure determination of a macromolecular assembly.

- In addition to supporting regional centers with cryo-EM research expertise and extensive wet-lab equipment and on-site sample preparation support, significant investment in local university and research institute cryo-EM infrastructure will need to be established nationwide to maximize the use of high-end national facilities.

- In addition, the development of publicly accessible software and provision of necessary computing resources will be needed.

Who are likely users of cryo-EM methods?

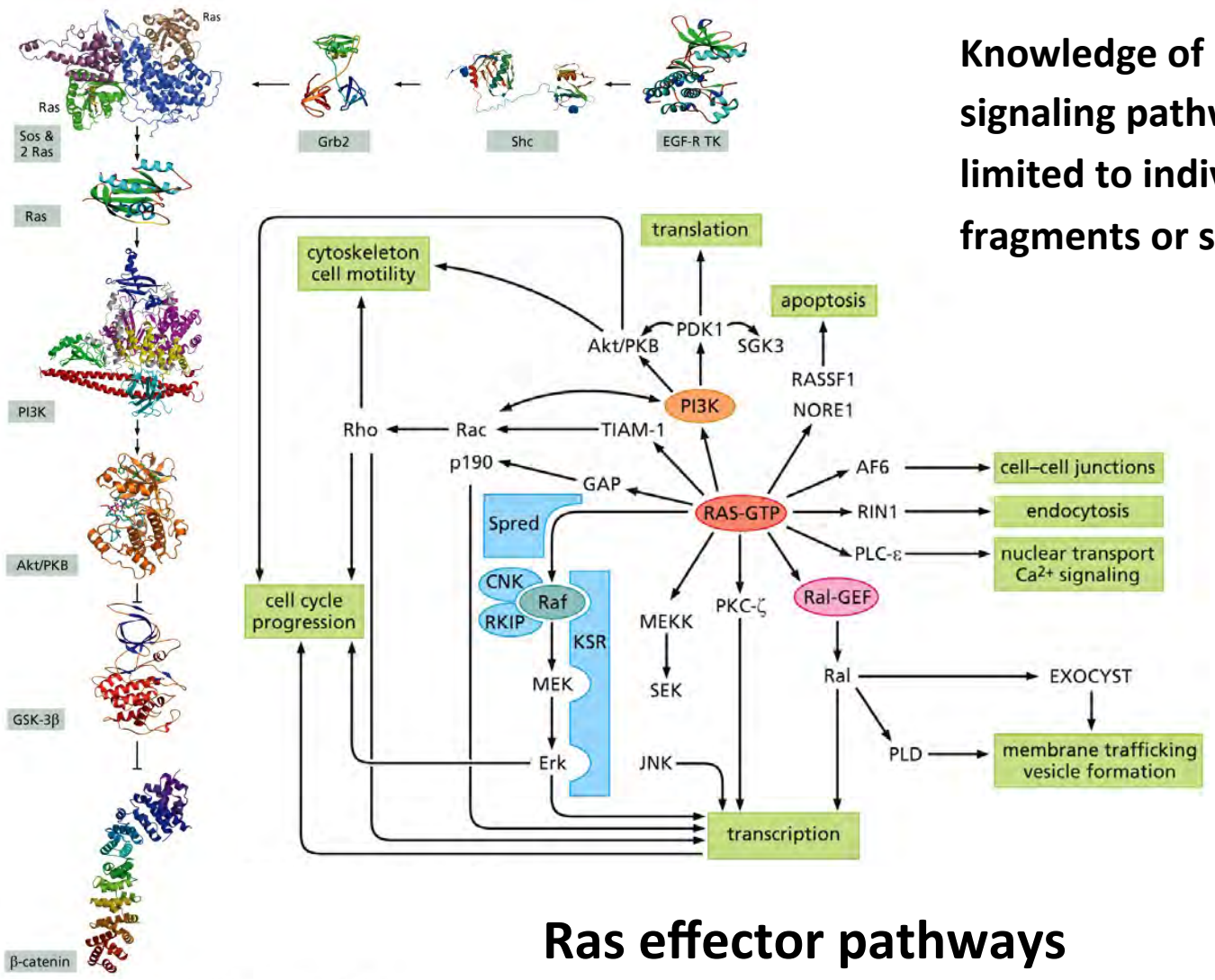
1. Cryo-EM specialists who need access to advanced instrumentation

2. Structural biologists, especially X-ray crystallographers and NMR spectroscopists who first need training, and then access to advanced instrumentation

3. Biologists who work on problems that could benefit from cryo-EM analyses, but need specialized training in all steps of the workflow

Gap analysis: The need for structural biochemistry

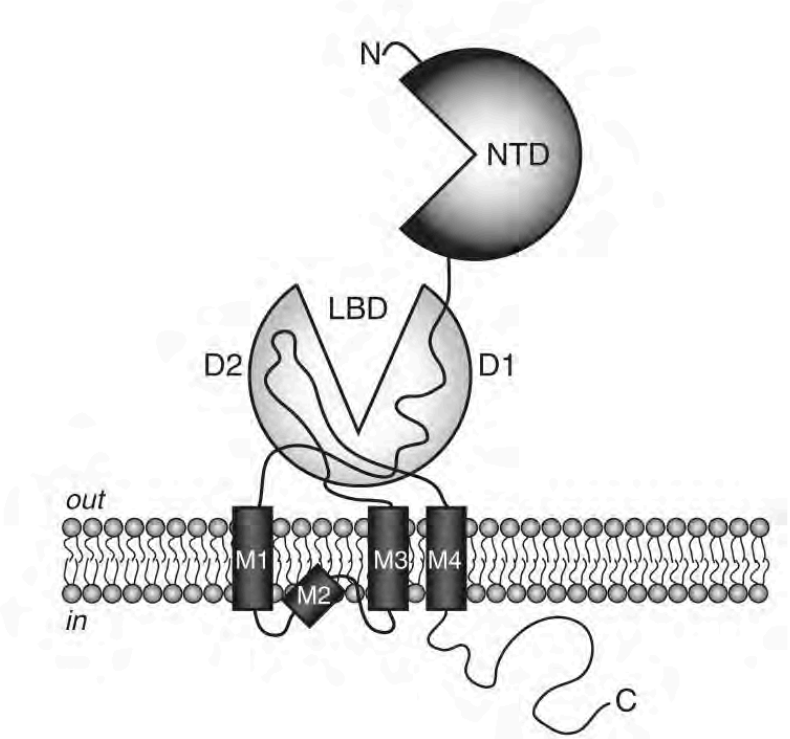
- All structural advances by cryo-EM so far have required close collaboration between electron microscopists and biochemists
- Each class of proteins has its own unique challenges
- Structure determination can be a slow, iterative process
- Without access to specialized knowledge at interface between biochemistry and EM, biologists may not be able to take advantage of the advances in the field.

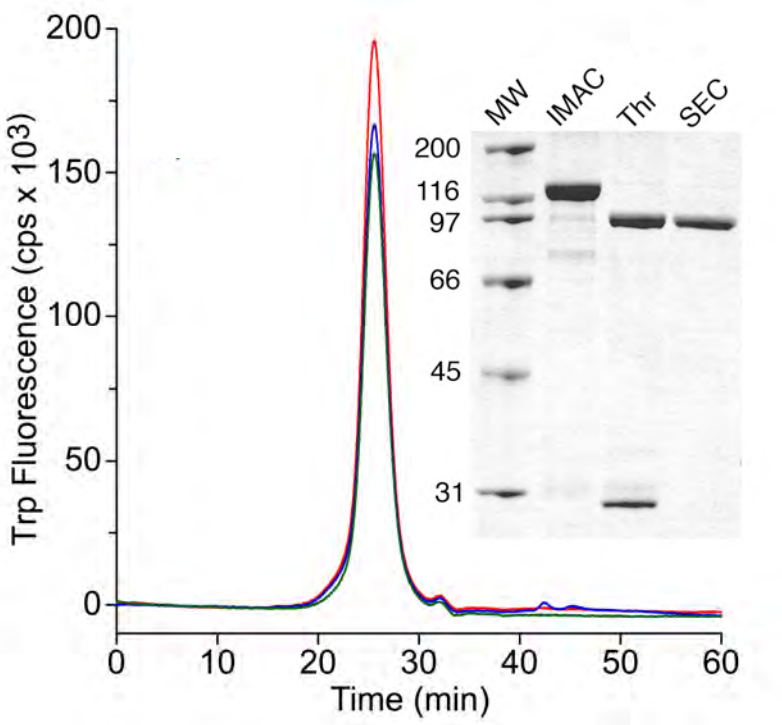


Knowledge of structures of signaling pathway is largely limited to individual protein fragments or small sub-complexes

Ras effector pathways

Cryo-EM structure of the glutamate receptor: A case study

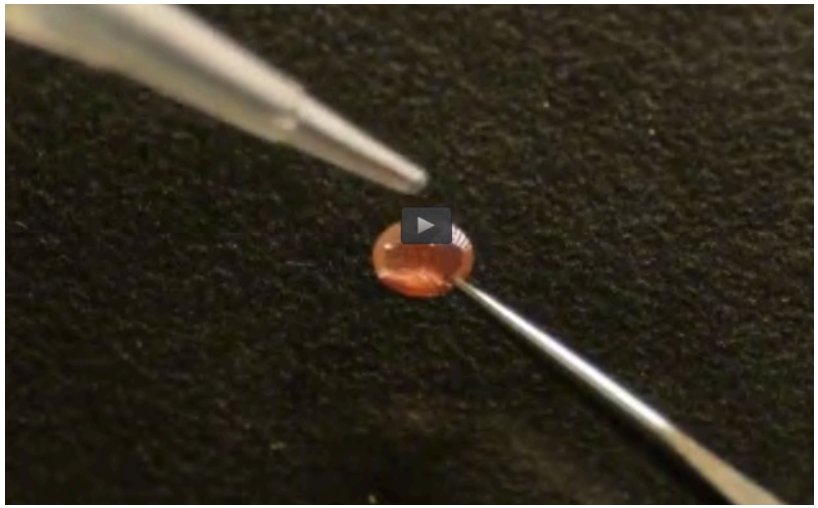


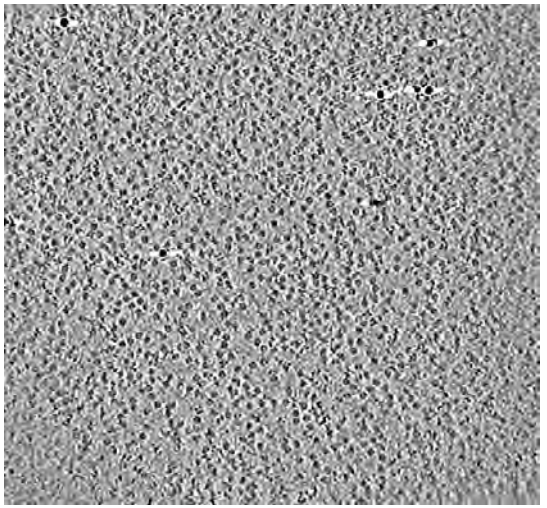


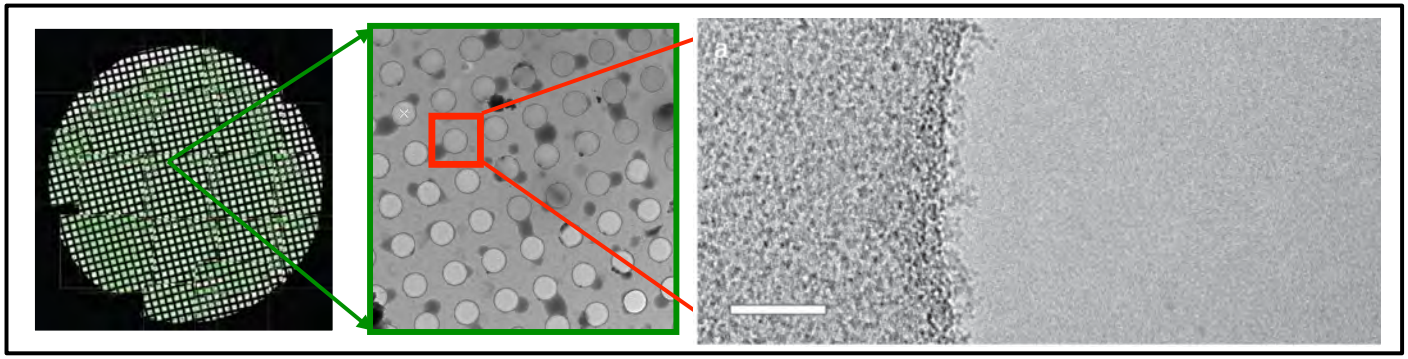
- **integral membrane protein (~ 400 kD)**
- **understanding gating cycle is of fundamental interest to drug design**

- **It took Mark Mayer and his laboratory about a decade to get the biochemistry and purification protocols worked out**

Challenges in preparing useful cryo-EM specimens from purified protein

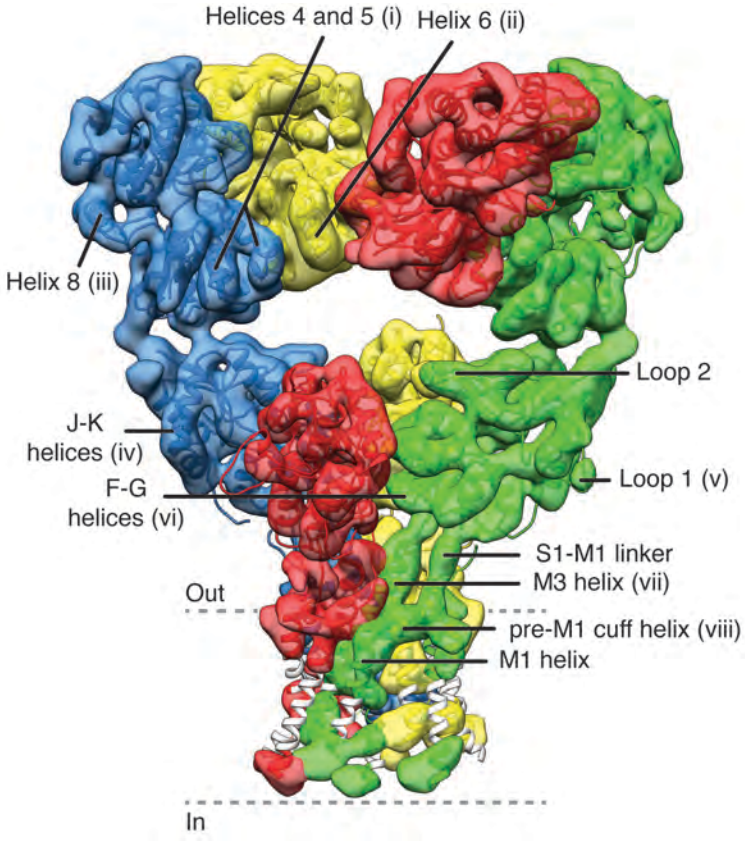


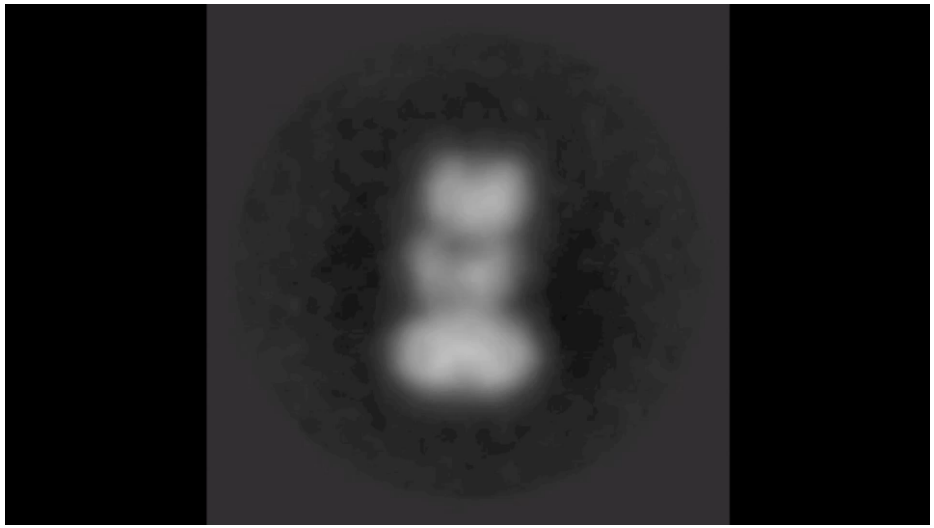




Proteins were supposed to go into the holes but they didn't, and ended up on the carbon

From structure at ~ 50 Å resolution to ~ 7 Å resolution





Meyerson et al *Nature* (2014)

Key expertise elements necessary for biologists to become users of cryo-EM technology

- Know-how and resources for state-of-the-art molecular biology
- Knowledge of diverse expression systems to produce proteins and relevant complexes with the correct post-translational modifications
- Effective purification strategies coupled with a broad spectrum of biochemical readouts of function and biophysical readouts of stability
- Expertise in reconstitution, especially for those complexes that are membrane-associated
- Expertise in cryo-EM image processing and structure determination,
- High-throughput screening to evaluate suitability for cryo-EM analyses
- Interest and competency for developing streamlined approaches for specimen preparation
- Extensive resources for high-speed computing and data storage

Unique features of FNLCR as a national center focused on structural biochemistry and high-throughput cryo-EM screening

- Active NCI CRADA with major cryo-EM manufacturer since 2012 aimed at developing automated workflows for small, dynamic protein complexes
- Outstanding biochemistry and biophysics infrastructure already present, with expertise in structural biology relevant to cancer signaling
- Strong local partnerships with intramural program, University of Maryland and CCR Center for Molecular Microscopy
- Can become a magnet for cancer biologists and pharma who are looking for collaborations and help to advance structural studies of cancer-relevant protein complexes

Summary for potential FNLCR cryo-EM facility

- Focus on converting biochemical samples relevant to important biological problems into specimens that can be used for high-resolution cryo-EM analysis
- Once conditions are worked out, there will be need for analyzing large numbers of related complexes that can be done at other user facilities or local institutions
- Will lower barrier for biologists to enter field
- Distinct mission from national user facilities
- Take on especially challenging projects such as Ras structural biology where there is great depth and breadth at FNLCR